



The Ups and Downs of Adenovirus Vectors

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Abstract. *Owing to the detailed knowledge of the structure of the adenovirus virions, including their DNA genomes, especially types 2 and 5, they are convenient viruses for construction of vectors for gene therapy and vaccine immunization. It is critical to note, however, that adenoviruses produce pathogenic inflammatory responses to infection. The inflammation occurs even if the adenovirus does not replicate when the inoculum is sufficiently large, because only early gene expression is responsible for the pathogenic reaction. The inflammation consists of an early phase, in which tumor necrosis factor alpha (TNF-alpha) plays a major role, and a late phase consisting of an extensive T-cell response. It is important in the construction of adenovirus vectors not to delete a major portion of the early region 3 (E3) because: the E3 19kD glycoprotein markedly reduces the capacity of the Class I major histocompatibility complex (Class I MHC) from transporting viral antigens to the surfaces of infected cells; and the E3 14.7 kD protein significantly inhibits the production of TNF-alpha and, therefore, reduces the polymorphonuclear response. Unfortunately the first generation of adenovirus gene therapy vectors contained large E3 deletions and, therefore, presented a significant safety problem. Subsequent adenovirus vectors consist of other deletions to overcome this difficulty.*

Although it still is in its early scientific phase, the viral vector has become an increasingly popular approach for the treatment of genetic diseases. One of the viruses that has been subjected to major vector analysis is the adenovirus. It has many advantages for vector investigations and possible clinical use.

Adenoviruses were discovered in 1953.^{1,2} These viruses are well known in terms of their double-stranded genomes, virion structure, and method of replication. *In vitro*, they grow to high titers, maintain their infectivity well, and are easy to handle. Adenoviruses, therefore, are relatively easy for vector construction. The

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vectors themselves can be replicated to high titer, so they also can be used readily. Moreover, the genes that are inserted in the appropriate places are well expressed. Adenoviruses are stable because of their protein structure. There are 47 human types, and the DNA genomes of several types have been well studied.³ Different types of vectors can be constructed readily.

Fundamentals of Vaccine Manufacture

Despite this level of knowledge, however, some misconceptions exist about viruses, particularly with regard to the making of vaccines. It seems to be generally believed that the only thing required to make a vaccine is to produce antibodies that attach, for example, to the adenovirus capsid fiber by which the virus attaches to susceptible cells. But in the case of the adenoviruses this is incorrect. Indeed, the adenovirus neutralizing epitope is on the outer surface of a capsid structure termed the hexon.⁴ It is important to note this fact because only one antibody molecule is required to neutralize a virus.⁵ However, if antibodies to the fiber were required to neutralize the viral infectivity, 60 such antibody molecules would be necessary to prevent attachment. This may be one reason that many of the vaccines against viruses have not been successful.

To understand the process of constructing vaccines or adenovirus vectors, one must begin with knowledge of how the virus replicates, and the structures of the viral genome and the virion proteins. Except for synthesis of viral proteins, viral replication occurs entirely in the nucleus of the cell. A number of early genes must be expressed before the DNA can replicate. Early regions 1A and 1B (E1A and E1B) play critical roles. E1A is important to activate transcription of each early gene efficiently.^{4,6} If mutations occur in E1A, the virus replicates very poorly except at high viral multiplicity of infection.⁶ In most of the adenovirus vectors, the new gene has been inserted in the deleted E1A and E1B regions because good gene expression can be obtained therein. It is critical to note that early region 3 (E3) is important in the pathogenesis of the virus and its life history. The E3 region is absolutely not

required for viral replication, and, therefore, it has been termed the nonessential region. In terms of the life history of the virus, however, the E3 region contains several very important gene functions for both the virus and the host.⁷⁻⁹

The E2A and E2B genes are essential for viral DNA synthesis. If DNA does not replicate, the late genes cannot be expressed, and the structural proteins will not be made.⁶

Early Construction and Application of Vectors

The first generation of adenovirus vectors was used for the treatment of cystic fibrosis.¹⁰ To produce the vector, the cystic fibrosis gene was inserted in place of the deleted E1A and E1B regions of the adenovirus DNA. In this vector construct (AdCFTR), the cystic fibrosis gene was very effectively expressed. To produce the vector, the E3 region was deleted, however, because it was considered to be nonessential.⁴ Indeed, to construct such a vector a portion of the viral genome must be deleted because no more than 105% of the original genome can be efficiently packaged into the virion procapsid.

If the vector is constructed properly, the gene has good expression and can infect many organs, including their dividing and nondividing cells. It is worth noting that retroviruses have been used even more extensively than adenoviruses, but they have one very grave deficiency: a dividing cell is required for infection and expression. Retroviruses, therefore, cannot be used effectively with many cells. There is a down side to the adenovirus vector as constructed, however, since a relatively severe inflammatory response occurs in the cotton rat animal model employed to test its efficiency and safety. Therefore, if inoculated into the lungs of humans for the treatment of cystic fibrosis, a pneumonia may be produced. Moreover, the period of expression in the cotton rat is limited, because of an immune response. In addition, numerous repeated infections with the vector over a very long period are also not possible because of the production of adenovirus neutralizing antibodies and probably cellular immunity.

Experiments studying the effect of wild-type Ad5 in cotton rats demonstrate the development of an inflammatory response (i.e., a

pneumonia) beginning 1 day after infection and reaching maximum pulmonary infiltration about 5 days after infection. The pneumonia consists of an infiltration in the alveoli early in infection, and an accumulation of lymphocytes around the bronchioles and bronchi in the later stage.^{11,12} When the adenovirus cystic fibrosis vector (Ad-CFTR) is used, the pneumonia is greatly increased owing to the extensive E3 deletion (unpublished data). Only early genes are required to produce the inflammatory response, and, therefore, the virus does not have to replicate; it only need express its early genes.⁹ This is an important point to note because the adenovirus vectors presently in use are replication defective. If the viral inoculum contains a sufficient number of viruses to give good gene expression, extensive pneumonia is produced in the infected animals. Some have termed this inflammation a toxic effect. We now have evidence, however, that the effect is not due to "toxicity" caused by the viral coat proteins: if one inactivates the virus with ultraviolet light the genes cannot function, but the proteins on the outside of the viral particles are still intact and immunologically active. Nevertheless, the UV-inactivated virus cannot produce pneumonia, proving that the viral early genes of the adenovirus are responsible for producing inflammation (unpublished data).

The early region 3 (E3) is important to retain in the vector because it protects the virus from important cell functions. An E3 19-kilodalton glycoprotein markedly reduces the Class I major histocompatibility complex (MHC I) from migrating to the cell surface with viral antigens and, therefore, markedly reduces the cytotoxic T cell response to virus-infected cells.⁷⁻⁹ At the other end of the E3 region, there is a 14.7-kilodalton protein that decreases Tumor Necrosis Factor-alpha (TNF-alpha) cytokine production. The tumor necrosis factor is very important in its production: if one deletes E3, as in the first generation of the adenovirus cystic fibrosis vector,¹⁰ the inflammatory response is markedly increased, because a very high proportion of the inflammation consists of cytotoxic T cells and polymorphonuclear infiltration. In the early phase of inflammation, between 1 and 5 days after the viral infection, the cytokines TNF-alpha, IL-1 and IL-6 are in-

duced.¹³ The major cytokine response inducing inflammation is caused by TNF-alpha. When antibodies to TNF-alpha are employed, the inflammation of the early phase decreases markedly. Antibodies to IL-1 do not decrease inflammation, and we have been unable to obtain good antibodies to IL-6. However, the late phase of this inflammation, which can be very severe, does not occur in nude mice, which do not have cytotoxic T-cells. IL-6 appears to be important in inducing a cytotoxic T-cell response (unpublished data).

We have made a second-generation vector by restoring all of the E3 region except for the region encoding the 11.6 and 10.4 kD proteins. This small region of the genome remains deleted to provide the necessary space to allow efficient packaging of the viral genome containing the cystic fibrosis gene. This viral vector reduces the inflammation significantly but, unfortunately, does not reduce it sufficiently to be considered a safe vector (unpublished data). This construct does suggest, however, that these E3 genes encoding the 11.6 and 10.4 kD proteins have other functions. Studies are now being pursued to determine whether they have functions in cytokine induction or as important antigens for cytotoxic T-cells to recognize and attack. Several other investigators are also developing viral mutations in an effort to reduce the vector-induced inflammation.¹⁴

We are presently constructing a vector in which E4 is deleted. Deletion of the entire E4 region reduces inflammation significantly, but at this stage one cannot be certain that it will serve as a safe vector in humans. An important aspect of our current research, therefore, is to discover which genes are inducing cytokine production and are necessary for the antigen production for the cytotoxic T-cell response.

Conclusions

Potentially, adenoviruses can be effective vectors if the inflammatory problem can be solved. Thus, we investigators must determine how to maintain a very low rate of cytokine induction or none at all, and to obtain a minimal response of virus-specific cytotoxic T-cells. Hence, we must determine all of the genes that

are responsible for cytokine induction and identify the viral antigens that induce the CTL response. The next generations of vectors must restore early region 3 but delete part of the genome for efficient packaging, perhaps early region 2 or early region 4.

The potential for adenovirus vectors is vast. In addition to their use for gene therapy, they are also being tested for production of vaccines and for therapy of malignancies.

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